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# Formulation and Physical Properties Observations of Soy Lecithin Liposome Containing 4-*n*-Butylresorcinol

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**Abstract.** Liposomes are preparations which have many advantages such as high solubility and good stability. The 4-*n*-butylresorcinol has the effect of hypopigmentation and, similar to resorcinol, is unstable. Liposomes could, therefore, assist the development of 4-*n*-butylresorcinol in a more appropriate dosage form. The research presented in this article aimed to determine the effect of the concentration of soy lecithin phospholipids to the liposome particle size. Subsequently, the effect of adding 4-*n*-butylresorcinol as the active compound to the liposomes in the particle size and morphology of the liposomes is also presented. The preparation of liposomes was assisted by sonication, and subsequently, the active compound 4-*n*-butylresorcinol was added. Particle size and morphology of liposomes were then characterized as the observed parameters. In this research, there was no evidence that the size of the liposomes was significantly affected by the concentration of phospholipids. Notably, the particle size and morphology of liposomes with 4-*n*-butylresorcinol as the active substance were not significantly different compared to the liposomes without 4-*n*-butylresorcinol.

## INTRODUCTION

Liposome is one of drug delivery systems which can be applied in various semisolid preparations with a variety of dosage forms such as gels, creams, and lotions. Liposome administration can be delivered by ocular, nasal, oral, intramuscular, subcutaneous, and topical. Lecithin consists of phospholipids which are often used in the manufacture and formulation of the liposome. This approach has several advantages including good stability against variations in pH or the concentration of salt in the formula; easily obtained [1], contains unsaturated fatty acids that provide relatively fluid and deformable properties to improve penetration through the epidermal barrier; biocompatible [2]. The liposome as a drug delivery system has advantages as a vesicular carrier. Liposomes also have the ability to protect the drug from the influence of the external environment using encapsulating the drug. Liposomes also have some disadvantages such as high cost, leakage encapsulation, the possibility of hydrolysis or oxidation of phospholipids that can have an impact on the stability of the medicine [3].

The particle size become one of the physical properties to be considered in liposomes preparation. Sonication is an approach to minimize the size of particles [4]. The average size of the resulting liposomes for drug delivery systems is 50-100 nm [5]. The quality of a liposome can be seen and measured with various parameters, such as liposome size and distribution and encapsulation efficiency (EE). The liposome size is influenced by several factors such as the concentration of phospholipids, the amplitude of sonication and sonication time [6]. The main constituent of phospholipid liposomes is amphiphilic molecules containing the head hydrophilic (water soluble) and a hydrophobic tail (lipid soluble). The preparation of liposomes with flexible membrane phospholipids can increase drug delivery through the skin compared to liposomes with phospholipid membranes rigid [7-8].

The method in liposomes preparations plays a role in determining the encapsulation of hydrophilic drugs [9-10]. The active compound 4-*n*-butylresorcinol has a hypopigmentation effect by inhibiting the activity of tyrosinase enzyme and suppress the synthesis of tyrosinase, which in turn inhibit the synthesis of melanin skin [11-12]. This study used 4-*n*-butylresorcinol as an active compound in the preparation of liposomes. The 4-*n*-butylresorcinol is a compound which has hydrophilic properties [13]. As a resorcinol derivative, this compound has instability in preparations characterized by discoloration caused by oxidation events [13]. Physical stability of 4-*n*-butylresorcinol can be improved by encapsulation of 4-*n*-butylresorcinol to the liposomes structures.

This research aimed to determine the effect of soy lecithin phospholipids concentration to the liposomes particle size and the effect of adding 4-*n*-butylresorcinol as the active compound to the liposomes particle size and morphology parameter.

## MATERIALS AND METHODS

Materials used in this study were: soybean lecithin (Sigma-Aldrich), distilled water, 4-*n*-butylresorcinol (SHREEJI Pharma International), phosphate buffer saline pH 7.4 (Biogear). The instruments used in this study were a blender, ultra turrax, sonicator, particle size analyzer (HORIBA Scientific, JAPAN), Transmission Electron Microscope (TEM), Spectrophotometer UV-VIS (Shimadzu, JAPAN).

Preparation of liposomes referred to the source [14-15] with some modifications. Liposomes were prepared by dispersing phospholipid in different concentrations (Table 1) in 100 mL distilled water 60 °C. Soy lecithin phospholipids and water were mixed in a blender for 60 seconds then were homogenized in an ultra turrax for 60 seconds. These mixtures were subjected further to sonication for about 30 minutes in 60 °C. The resulting liposomes subsequently were analyzed using a particle size analyzer to examine the particle size and Transmission Electron Microscopy (TEM) to see the morphology of the liposomes [16].

TABLE 1. Phospholipid concentration in sample

Sample	Phospholipid concentration (gram)	Distilled water (mL)
1	12.04	100
2	8.70	100
3	6.81	100
4	5.59	100
5	4.74	100
6	4.12	100
7	3.64	100
8	3.26	100

The active compound 4-*n*-butylresorcinol (0.10 % w/v) was added into liposomes which were prepared using 8.70 g soy lecithin phospholipid (Sample 2 in Table 1). Compound 4-*n*-butylresorcinol in liposomes was then estimated by centrifugation method [17]. Together with liposomes (0.50 mL), 1.00 mL phosphate buffer (pH 7.4) were placed in centrifugation tube and centrifuged at 14000 rpm for 30 minute [17]. The supernatant (250 µL) was then diluted with phosphate buffer (pH 7.4) until 10.00 mL. The 4-*n*-butylresorcinol encapsulation was determined by UV spectrophotometer at 278.5 nm. The free 4-*n*-butylresorcinol in the supernatant was defined as the total amount of not encapsulation drug (free drug). Total drug concentration was assumed as the added active substance, which is 0.1 % w/v. Encapsulation efficiency (EE) 4-*n*-butylresorcinol expressed as the percent of the trapped drug. The %EE equation is therefore as follows: %EE = ((total drug – free drug) x 100%)/total drug.

## RESULTS AND DISCUSSION

The results of liposomes size measurement are presented in Table 2. One way ANOVA showed that the liposomes sizes were not affected significantly by the concentration of soy lecithin phospholipids (*p*-value = 0.346). These results were not in line with the results of Nursaiah *et al.* [6], which reported that phospholipids concentration (lecithin and phosphatidylcholine) (*p* < 0.05) influenced the size of nanoliposomes significantly. The nanoliposomes size was observed to increase with the increase of the phospholipids concentration [6]. These differences with Nursaiah *et al.* [6] could be caused by the different range concentrations compared to this research. Nursaiah *et al.* employed 0.1-0.3% w/v phospholipid concentration [6], while this research used 3.26 – 12.04 % w/v phospholipids concentration. Interestingly, the diameter of liposomes resulted from this research was much smaller

(90.07–102.73 nm) compared to the results from Nursaiah *et al.* ( $228 \pm 48.6$  nm) [6]. Phosphatidylcholine is the main phospholipid from soybean lecithin [18]. Risselada and Marink investigated the effects of temperature and composition of the membrane structure and dynamic properties of the liposome with a diameter of 15-20 nm using a method of coarse-grained molecular dynamics simulations and shown that there is the effect on the composition of the membrane with dipalmitoyl phosphatidylcholine (DPPC) to liposomes size [19]. Interestingly, this effect not observed in polyunsaturated dilinoleylphosphatidylcholine (DLiPC). Notably, soy lecithin contains both of them. Therefore, the results here were in line with Risselada and Marink investigation. Further analysis computational methods could hopefully reveal the mechanism at the atomic level [20].

**TABLE 2.** The size of liposomes in various phospholipids concentration

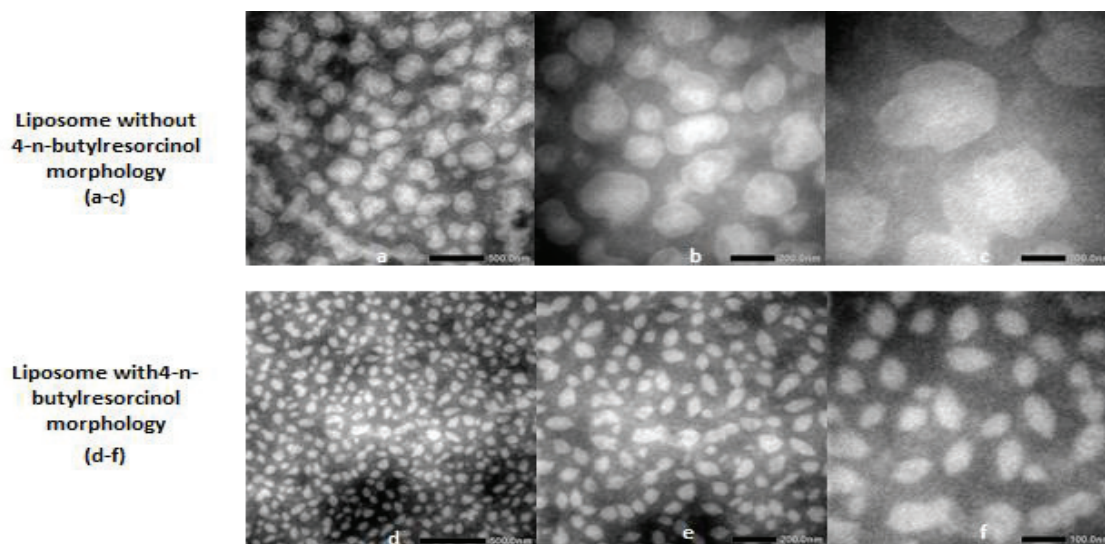
% Phospholipid	Liposomes size (nm)				
	I	II	III	average	SD
120.40 mg/mL	102.27	94.42	93.65	96.78	4.77
87.00 mg/mL	97.97	92.04	81.80	90.60	8.18
68.10 mg/mL	98.81	91.06	99.07	96.31	4.55
55.90 mg/mL	102.18	95.81	96.65	98.21	3.46
47.40 mg/mL	101.86	92.01	90.21	94.69	6.27
41.20 mg/mL	110.07	103.73	94.23	102.68	7.97
36.40 mg/mL	114.87	90.95	102.36	102.73	11.96
32.60 mg/mL	99.97	75.05	95.18	90.07	13.22

Liposomes could be a suitable preparation as a carrier 4-*n*-butylresorcinol compound. Liposomes could improve the stability of 4-*n*-butylresorcinol, which is easily oxidized and affecting the physical properties of the preparations. The effect of 4-*n*-butylresorcinol on liposomes size is presented in Table 3. Encapsulation compound 4-*n*-butylresorcinol in liposomes aimed to improve the physical stability and penetration of the active substance [21]. T-test results of measurement showed that the liposomes size without 4-*n*-butylresorcinol was not different significantly with liposome contains 4-*n*-butylresorcinol (p-value = 0.286).

**TABLE 3.** The effect of 4-*n*-butylresorcinol on liposomes sizes

Sample	Liposomes without 4- <i>n</i> -butylresorcinol size (nm)	Liposomes contain 4- <i>n</i> -butylresorcinol size (nm)
1	98.11	100.46
2	97.88	105.73
3	103.12	101.56
average	99.70	102.58
SD	2.96	2.78

Morphological description details of liposomes with and without 4-*n*-butylresorcinol observed using TEM are presented in Fig. 1. The 4-*n*-butylresorcinol as the active substance on liposome structure did not affect the sizes of the liposomes. Notably, the morphological description of liposomes with 4-*n*-butylresorcinol was seen more rigid compared to the liposomes without 4-*n*-butylresorcinol. Cavities were identified in the liposomes without 4-*n*-butylresorcinol.



**FIGURE 1.** Liposomes morphology

The %EE ( $\pm$  SD) 4-*n*-butylresorcinol in liposomes was 76.45% ( $\pm$  0.354). This result indicates that 4-*n*-butylresorcinol could be encapsulated by the liposomes preparations system. Therefore, it is expected that it could improve the physical stability of 4-*n*-butylresorcinol.

## CONCLUSION

In the liposomes preparations using soy lecithin phospholipids, the particle size was not significantly affected by the concentration of the phospholipids and by the encapsulation of 4-*n*-butylresorcinol as an active substance. However, the encapsulation of 4-*n*-butylresorcinol resulted in different liposomes morphology compared to the liposomes without 4-*n*-butylresorcinol.

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